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## Anthrax attack bug "identical" to army strain

19:00 09 May 2002 by Debora MacKenzie

The DNA sequence of the anthrax sent through the US mail in 2001 has been revealed and confirms suspicions that the bacteria originally came from a US military laboratory.

The data released uses codenames for the reference strains against which the attack strain was compared. But **New Scientist** can reveal that the two reference strains that appear identical to the attack strain most likely originated at the US Army Medical Research Institute for Infectious Diseases at Fort Detrick (USAMRIID), Maryland.

The new work also shows that substantial genetic differences can emerge in two samples of an anthrax culture separated for only three years. This means the attacker's anthrax was not separated from its ancestors at USAMRIID for many generations.

The new genetic sequencing work was done by the Institute for Genomic Research in Rockville, Maryland (TIGR), and Paul Keim's team at the University of Northern Arizona at Flagstaff. Before the attacks, TIGR had started sequencing a non-pathogenic derivative of the "Ames" strain of anthrax from the UK biodefence establishment at Porton Down.

It happened that the anthrax attacker used a pathogenic Ames strain. So in January, TIGR added the bacteria isolated from the first victim of the attack, Florida journalist Robert Stevens, to its sequencing effort.

### Incriminating evidence

The idea was to tease out subtle differences between the two genomes that might identify the source of the attack strain. Full-blown sequencing seemed necessary, as genetic differences in anthrax are notoriously hard to find.

The teams found plenty of differences between the two strains, as they now report in the journal *Science*. They then took these "marker" stretches of DNA and tested them against five other samples of Ames anthrax, looking for differences - or incriminating similarities.

One, from a goat that died of anthrax in Texas in 1997, differed at four markers, proving that the markers can reveal divergence among anthrax lineages.



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But ironically, none of the other four - identified only as A, B, C and D - differed at all from the attack strain at any of the new markers revealed by sequencing. However, two, A and D, did differ at one marker - a stretch of repeated adenines on pXO2, one of the two DNA plasmids that give anthrax its virulence.

That marker had already been discovered by Keim and reported at a meeting in June 2001. "It may be the most polymorphic site in the genome," Keim told **New Scientist**. Strain A can immediately be ruled out as the attack strain as it is missing a plasmid, and is non-pathogenic.

The identity of the strains apparently identical to the attack strain - B and C - and strain D can be deduced as follows. In February, Keim told **New Scientist**: "We can distinguish among different Ames accessions. These are from collaborative laboratories and related to genetic work we have been performing over the years."

Doubly sure

The strains from the collaborative labs appear certain to be strains B, C and D. In that case, one was the reference Ames in Keim's collection that came from a freezer at Porton Down, which in turn had got it from USAMRIID. Another was a culture that came directly from USAMRIID, and the last was from the US Army's Dugway proving ground in Utah.

TIGR spokesmen and other sources have stated that Keim could find no differences between the attack strain and the reference Ames in his collection at any marker tested in his lab. The tests reported in *Science* are no better at doing this. So one of B and C is Keim's Porton Down/USAMRIID reference strain. The other is likely to be the culture directly from USAMRIID, as the reference strain originated there and had since languished in a freezer.

So strain D seems to have come from Dugway. The difference between D and the attack strain is not great - there are 36 adenines in a row, instead of 35 - but Keim's team made doubly sure by sequencing that part of the D strain's genome.

However, the new work does not prove irrefutably that the attacker got his anthrax directly from USAMRIID because it is possible that untested Ames cultures from other labs might also be identical. Those tests are now underway.

Journal reference: *Science* (DOI: 10.1126/science.1071837)

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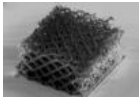


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